

AUTONOMIC NEUROPATHY IN DIABETES MELLITUS

**WITH SPECIAL
REFERENCE TO TEST PROCEDURES,
PERIPHERAL CIRCULATION,
AND ENDOTHELIAL FUNCTION**

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This thesis is based on the following papers, referred to by Roman numerals in the text.

- I Sundkvist G, Almér L-O, and Lilja B: Respiratory influence on heart rate in diabetes mellitus. Br Med J I:924-925, 1979.
- II Sundkvist G, Lilja B, and Almér L-O: Abnormal diastolic blood pressure and heart rate reactions to tilting in diabetes mellitus. Diabetologia 19:433-438, 1980.
- III Sundkvist G: Autonomic nervous function in asymptomatic diabetic patients with signs of peripheral neuropathy. Diabetes Care 4:529-534, 1981.
- IV Sundkvist G, Lilja B, and Almér L-O: Deep breathing, Valsalva, and tilt table tests in diabetics with and without symptoms of autonomic neuropathy. Acta Med Scand (in press), 1982.
- V Almér L-O, Sundkvist G, and Lilja B: Endothelial factors, toe temperature and leg circulation in diabetics with and without autonomic neuropathy. Thrombosis Res (in press), 1982.



Deep Breathing, Valsalva, and Tilt Table Tests in Diabetics with and without Symptoms of Autonomic Neuropathy

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ABSTRACT. Autonomic neuropathy (AN) test results (Valsalva manoeuvre, deep breathing, and tilting) are frequently impaired in diabetics without symptoms of AN, particularly in those with peripheral neuropathy (PN). We have investigated 24 asymptomatic diabetics with PN, 17 diabetics with symptoms of AN and 24 healthy controls. The heart rate reactions to the three tests were impaired in both patient groups. The Valsalva manoeuvre could not distinguish between the patient groups. The heart rate reaction to deep breathing, estimated as the expiration/inspiration (E/I) ratio, was slightly more disturbed in patients with AN than in those with PN (1.04 vs. 1.09, $p < 0.05$) but the frequency of abnormally low E/I ratios was high in both groups (76.5 vs. 54.2%, NS). The immediate heart rate reaction to tilting, estimated as the brake index, clearly separated the patient groups. An abnormally low brake index was shown in 82.3% of AN patients and in 33.3% of PN patients ($p < 0.01$). The study shows that the deep breathing test is sensitive for AN but an impaired immediate heart rate reaction to tilting is more specific for symptomatic AN.

Key words: diabetic neuropathy, autonomic nervous system, postural hypotension, deep breathing, Valsalva manoeuvre, orthostatic test, tilt table.

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Various autonomic neuropathy (AN) tests have been introduced during the last decade (2). Deep breathing tests, the Valsalva manoeuvre and orthostatic procedures have been found to be most useful (5, 8, 24, 25). In 1979 we found that the heart rate reaction to deep breathing was frequently impaired in diabetics without symptoms of AN (23), most commonly in those with peripheral neuropathy (PN) who also frequently demonstrated orthostatic disturbances (22). Thus there is an in-

crease in the extent as well as in the degree of abnormalities when signs of PN are present. Symptoms of AN, regarded as a serious complication (6, 7, 19), are well known in diabetics (20). Whether such patients show aggravated test abnormalities is unknown.

The aim of this study was to find out if there are differences in the AN test results between diabetics with just PN and diabetics with symptoms of AN as well.

SUBJECTS

Forty-one insulin-dependent diabetic patients and 24 healthy volunteers were investigated. Informed consent was obtained from all. The patients were on no other medication than insulin and showed no evidence of cardiac failure. All subjects were tested for PN by analysis of tendon reflexes and vibration sense (Bio-Thesiometer) (22).

AN group comprised 17 diabetics (age 32–76 years, mean 56, 3 females) selected from a diabetic clinic because of symptoms of AN reported in a questionnaire (Table I). Incomplete impotence was not considered a symptom of AN. Fifteen patients had signs of PN (vibration sense threshold values on the medial malleoli above the mean (+2 S.D.) of the controls and/or absent ankle reflexes). The duration of diabetes was 9–43 years (mean 24).

PN group comprised 24 age-matched diabetics (age 35–74 years, mean 52, 12 females) selected from a diabetic clinic. They had signs of PN but no symptoms of AN. The mean duration of diabetes was 30 years (range 12–49) and did not differ significantly from that in the AN group (t -test).

C group comprised 24 age-matched control subjects (age 40–74, mean 53, 8 females) who had undergone a health examination.

Abbreviations: AN = autonomic neuropathy, PN = peripheral neuropathy, ECG = electrocardiogram, E/I ratio = expiration/inspiration ratio, BP = blood pressure.

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Table I. Prevalence of symptoms of AN

Symptom	1 symptom (n=11)	2 symptoms (n=5)	3 symptoms (n=1)	Total
1. Postural	6	3 ^c	1	10
2. Gastrointestinal ^a	2	0	1	3
3. Gustatory sweating	0	3 ^d	0	3
4. Miction disturbance ^b	0	1	0	1
5. Total impotence	1	0	0	1
6. Ejaculatory dysfunction	2	3	1	6

^a Otherwise unexplained frequent vomiting or nocturnal diarrhea.

^b Voiding difficulties due to a large atonic bladder.

^c 1+3, 1+4 and 1+6.

^d 1+3, 3+6 and 3+6.

METHODS

Deep breathing test. After 15 min in the supine position the subject's heart rate was checked for at least 4 min and, once constant, a continuous ECG was recorded during a deep breathing procedure lasting for 1 min (23) with 6 maximal expirations and inspirations. The E/I ratio was calculated from the mean values of the longest R-R interval during expiration (E) and the shortest during inspiration (I).

The Valsalva manoeuvre. After another 10 min of rest the subject performed a Valsalva manoeuvre (5) by blowing through a mouth-piece attached to a manometer and maintaining a pressure of 40 mmHg for 15 sec. Once the technique had been mastered by the subject, the test was performed 3 times during continuous ECG recording. Between each blowing, the subject rested for 1 min. The Valsalva ratio was calculated from the 3 ECG recordings (the mean longest R-R interval after the manoeuvres to the mean shortest during the manoeuvres). Two patients with symptoms of AN were not able to perform the Valsalva manoeuvre.

Orthostatic test. After the Valsalva manoeuvre the subject was fastened to a tilt table. To start with, the table was tilted head up in order to familiarize the subject with the procedure. After that the subject was returned to the supine position for 10 min and was then tilted to the upright position (90°) in 2 sec and remained so for 10 min. ECG was recorded continuously during the orthostatic test with start 1 min before tilting. The ECG signals were processed in a computer (ABC 80, Luxor, Motala, Sweden) for registration of R-R intervals and time. Systolic and diastolic blood pressures (BP) were recorded indirectly in the right arm with a sphygmomanometer 4 min before tilting and every minute thereafter.

Fig. 1 shows the R-R intervals before and after tilting. The R-R intervals were measured during the first 30 sec of the last minute before tilting and the mean value was taken as the R-R interval at rest (A). During the first minute after tilting the shortest R-R interval (B) before the transient period of prolonged R-R intervals was selected as well as the longest (C) after B. The R-R intervals were then determined every minute. To further clarify the initial changes, we estimated the acceleration index $((A-B)/A \times 100)$ and the brake index $((C-B)/A \times 100)$ (24). To calculate the acceleration index in subjects with a brake

index = 0, i.e. those without a deceleration, the shortest R-R interval during the first 20 sec after tilting (time for shortest R-R interval in the controls + 2 SD) was selected and used as B for estimation of the acceleration index $((A-B)/A \times 100)$.

Statistical analysis. Ratios and indices were analysed by Mann-Whitney *U*-test because of non-normal distribution. The prevalence of abnormal findings was tested by Fisher's exact probability test. Otherwise, unpaired *t*-test was used. All tests were two-tailed. The null hypothesis was rejected when $p < 0.05$. Results are given as mean $\pm$ SEM.

RESULTS

Control subjects

Table II shows the results of the deep breathing test and Valsalva manoeuvre. The immediate heart rate reaction after tilting was characterized by a biphasic response which comprised an initial acceleration (shorter R-R intervals) followed by a transient deceleration (longer R-R intervals) (Fig. 1). The acceleration and brake indices measure these changes (Table II). Diastolic BP rose in controls after tilting (Table III).

Ratios or indices below the range of the controls as well as BPs outside the range of the controls were considered abnormal (Table IV).

Patients with PN

The mean E/I and Valsalva ratios were significantly lower and the supine systolic BP significantly higher in patients with PN than in controls (Tables II and III). After tilting the transient period of prolonged R-R intervals could scarcely be seen in patients with PN (Fig. 1). The mean brake index was significantly lower in patients with PN than in controls (Table II). The immediate acceleration was also impaired and the mean acceleration index was significantly lower in patients with PN than in con-

Table II. Heart ratios and indices in controls (C), diabetic patients with peripheral neuropathy (PN) and diabetic patients with symptoms of autonomic neuropathy (AN) (mean $\pm$ SEM)

	C (n=24)	Diabetics with		Significance		
		PN (n=24)	AN (n=17)	C vs. PN	C vs. AN	PN vs. AN
E/I ratio	1.23 $\pm$ 0.02	1.09 $\pm$ 0.01	1.05 $\pm$ 0.01	<0.001	<0.001	<0.05
Valsalva ratio	1.60 $\pm$ 0.06	1.44 $\pm$ 0.08	1.24 $\pm$ 0.06 ^a	<0.05	<0.001	NS
Acceleration index	17.0 $\pm$ 1.5	11.2 $\pm$ 1.4	5.9 $\pm$ 1.4	<0.01	<0.001	<0.05
Brake index	14.2 $\pm$ 1.8	5.3 $\pm$ 0.9	1.3 $\pm$ 0.6	<0.001	<0.001	<0.01

^a n=15.

trols. Diastolic BP fell 1 min after tilting in patients with PN (Table III).

Patients with AN

After tilting the initial acceleration and deceleration phases almost vanished (Fig. 1). All mean ratios and indices were significantly lower in patients with AN than in controls (Table II) and the mean systolic and diastolic BPs before tilting were significantly higher (Table III). After tilting, both systolic and diastolic BPs fell in patients with AN. All ratios and indices were abnormal in the patient with total impotence.

Comparisons between patients with PN and patients with AN

The mean E/I ratio as well as the acceleration and brake indices were significantly lower in patients with AN (Table II). The prevalence of an abnormal brake index was significantly higher in these patients (Table IV). The acceleration and/or the brake index were abnormal in 15 (88.2%) of the patients with AN and in 10 (41.7%) of those with PN ($p<0.01$). All AN patients with abnormally high systolic BP in the supine position showed an abnormal fall in systolic BP after tilting (Table IV). Such a fall was not seen in the only PN patient with abnormal supine recording.

DISCUSSION

This study shows that diabetic patients with PN but without symptoms of AN may show impairments in any of the tests, most frequently in the deep breathing procedure. Diabetics with symptoms of AN showed more pronounced test deviations. The most clear-cut difference between the patient groups was found in the orthostatic test which gave a high prevalence of abnormal indices, particularly the brake index, in those with symptoms of AN. Apparently,

the orthostatic test is most specific for symptomatic AN. On the other hand, the deep breathing test seems to be most sensitive for AN as also recently shown by Mackay et al. (16).

The heart rate reactions to deep breathing, here estimated by the E/I ratio, as well as to tilting, estimated by the brake index, are controlled by vagal nervous tone (24).

The immediate acceleration, here estimated by the acceleration index, has earlier also been attributed solely to changes in vagal tone (18). Recently, however, evidence has been presented favouring an additional sympathetic influence which increases the heart rate during the first 30 beats in the vertical

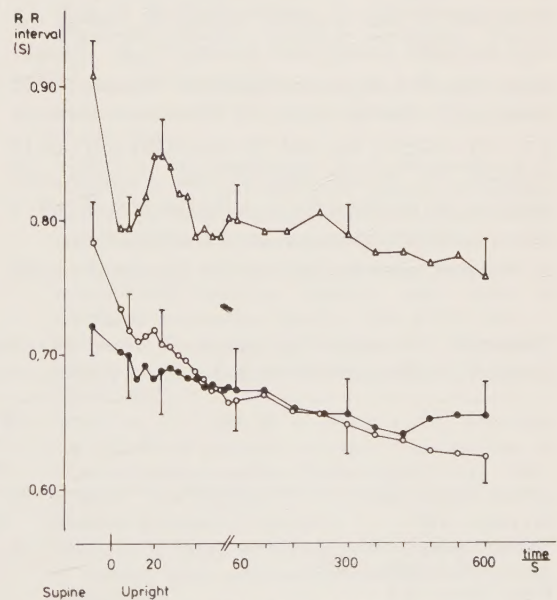


Fig. 1. Changes in heart rate during the orthostatic test in 24 controls (Δ), 24 diabetic patients with peripheral neuropathy ($\circ$) and 17 diabetic patients with symptoms of autonomic neuropathy ($\bullet$). Vertical bars = 1 SEM.

Table III. Blood pressures before and changes after tilting in controls (C), diabetic patients with peripheral neuropathy (PN) and diabetic patients with symptoms of autonomic neuropathy (AN) (mean $\pm$ SEM)

BP (mmHg)	C (n=24)	Diabetics with		Significance		
		PN (n=24)	AN (n=17)	C vs. PN	C vs. AN	PN vs. AN
Systolic, at rest	122 $\pm$ 3	140 $\pm$ 4	157 $\pm$ 6	<0.01	<0.001	<0.05
Δ systolic _{1 min}	-2 $\pm$ 2	-8 $\pm$ 3	-19 $\pm$ 8	NS	<0.05	NS
Δ systolic _{1-10 min}	-2 $\pm$ 2	-2 $\pm$ 3	-9 $\pm$ 6	NS	NS	NS
Diastolic, at rest	76 $\pm$ 2	80 $\pm$ 2	89 $\pm$ 4	NS	<0.01	<0.05
Δ diastolic _{1 min}	6 $\pm$ 1	-2 $\pm$ 1	-7 $\pm$ 3	<0.001	<0.001	NS
Δ diastolic _{1-10 min}	0 $\pm$ 1	2 $\pm$ 1	-1 $\pm$ 2	NS	NS	NS

position when the primary vagal mechanism is blocked (9). This is firmly supported by the present study. Our AN patients had predominating symptoms of sympathetic denervation and showed an impaired immediate acceleration.

The Valsalva ratio has been launched as a suitable test for AN (5) but here it could not distinguish between patients with symptoms of AN and those with PN only. The Valsalva test was originally introduced to measure myocardial function (15) and is still regarded as a reliable test of myocardial performance (25). Myocardial dysfunction is closely correlated with the duration of diabetes as well as to microangiopathy (21). The high frequency of abnormal Valsalva ratios among diabetics with symptoms of AN in earlier studies as well as the high mortality among such patients (5, 6, 7) might have been due to myocardial heart disease rather than to AN. Furthermore, the Valsalva manoeuvre is a very complex test and the results are difficult to evaluate (4). In our opinion, the Valsalva manoeuvre can be discarded as a reliable test of AN in diabetics with suspect myocardial dysfunction.

A conspicuous finding was the increased supine

BP in the diabetics. Baroreflex sensitivity is reduced in hypertension (10), and non-diabetics with borderline hypertension have reduced heart rate variations to deep breathing (12). The high BPs may have contributed to the heart rate deviations in the tests. The systolic BP fell after tilting to abnormally low values in all AN patients with abnormally high supine recordings. Those with PN only did not show such a reaction. The mean diastolic BP fell, however, in AN patients as well as in those with PN only. Falls in BP after rising are usually not seen in untreated hypertensive patients without diabetes mellitus (1). The BP reactions in the diabetics favour the concept of a baroreflex defect that also well explains the impaired acceleration after tilting. A similar defect in the acceleration is also present in elderly non-diabetics with idiopathic orthostatic hypotension (26).

Judging from our study, AN seems mostly to be an aggravation of PN although two patients without PN had AN symptoms. Vagal neuropathy is closely correlated with PN (22, 23), but vagal symptoms are apparently rare, like in our patients. Vagal neuropathy has, however, also other effects, such

Table IV. Prevalence of abnormal heart rate indices and blood pressures in diabetic patients with peripheral neuropathy (PN) and diabetic patients with symptoms of autonomic neuropathy (AN)

	PN (n=24)		AN (n=17)		Significance (PN vs. AN)
	No.	%	No.	%	
E/I ratio <1.09	13	54.2	13	76.5	NS
Valsalva ratio <1.18	7	29.2	6	40.0 ^a	NS
Acceleration index <5.2	5	20.8	9	52.9	<0.05
Brake index <3.8	8	33.3	14	82.3	<0.01
Systolic BP at rest >165 mmHg	1	4.2	6	35.2	<0.05
Δ systolic BP 1 min after tilting <-15 mmHg	6	25.0	8	47.1	NS
Diastolic BP at rest >90 mmHg	3	12.5	5	29.4	NS
Δ diastolic BP 1 min after tilting <-10 mmHg	1	4.2	5	29.4	NS

as impaired responses of glucagon and pancreatic polypeptide to hypoglycemia (14, 17). The heart rate reaction to exercise is also abnormal (11), and the heart rate at rest is increased (23, 25). The increased heart rate raises the heart rate-BP product indicating an increased myocardial oxygen consumption (13). Another possible myocardial metabolic consequence of cardiac denervation could be impaired glycolysis (3).

We conclude that vagal neuropathy is characterized by disturbed heart rate reactions to deep breathing and tilting. Sympathetic denervation is initially characterized by an absent rise in diastolic BP after tilting. Progress in sympathetic denervation inhibits further the immediate heart rate acceleration after tilting, and significant falls in systolic BP appear in the vertical position.

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ENDOTHELIAL FACTORS, TOE TEMPERATURE AND LEG CIRCULATION IN DIABETICS WITH
AND WITHOUT AUTONOMIC NEUROPATHY

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ABSTRACT

A low plasminogen activator response to venous occlusion is frequently found in diabetes. The aims of this investigation were to study any relationships between plasminogen activator activity and autonomic neuropathy (AN), and between abnormal toe temperature reactions and AN. Asymptomatic AN is frequently found in insulin dependent diabetics. The results might be an abnormal balance between the parasympathetic and sympathetic innervation of the vessels, which in turn will lead to a change of the reaction to cooling of the feet followed by indirect heating. In this study 52 insulin dependent diabetics were examined with a combination of tests for AN and blood flow measurements as well as plasminogen activator activities of the blood and vascular walls. In patients with a short duration of diabetes (mean 11 years) the prevalence of AN was high in those with abnormally slow increase in toe temperature after cooling followed by indirect heating. The mechanism behind appeared to be a functional vasospasm. In diabetics of short as well as of long duration (mean 35 years), an abnormally low plasminogen activator activity of the blood during venous occlusion was found in those without AN, while those with AN showed a normal activity. Thus, AN might influence the deterioration of the circulation in diabetes.

INTRODUCTION

In 1966 Moorhouse et al (1) found abnormal vascular reactions to thermal stimuli in the feet of diabetics with peripheral neuropathy (PN). The finding was supposed to indicate increased vascular reactivity owing to sympathetic denervation. Accordingly, autonomic neuropathy (AN) might contribute to foot lesions in diabetics with PN.

Key words: diabetic autonomic neuropathy, fibrinolytic activity, plasminogen activator, factor VIII, macroangiopathy, plethysmography, thermography

Vascular organic occlusions are also important for the development of diabetic foot lesions (2). The plasma concentration of the factor VIII related antigen (VIIR:Ag), which is most important for the platelet adhesion to vascular walls (3), is often high in diabetics (4). The plasminogen activator activity in the blood, which is essential for an adequate fibrinolytic function is low in many diabetics (5). Accordingly, in diabetes there is often a disturbed balance between coagulation and fibrinolysis, which favours the development of vascular occlusions. We have recently reported an almost normal fibrinolytic response to venous occlusion in diabetics with abnormal toe temperature reactions after cooling followed by indirect heating (6). AN mediated vasospasm was supposed to explain this finding.

The aim of the present study was to evaluate the influence of AN on leg circulation in diabetics, and especially to see whether there was a relationship between signs of AN and abnormal toe temperature reactions. Another aim was to study any relationships between AN on one hand and the plasminogen activator activity and VIIR:Ag on the other.

MATERIAL AND METHODS

Subjects

Patients. Fifty-two insulin dependent (Type I) diabetics were studied. A detailed report about the patients as well as control group 1 has been given (7). The patients were on no medication apart from insulin and came from a diabetic clinic comprising about 2000 patients. All patients were without evidence of cardiac failure, renal insufficiency, intermittent claudication or foot lesions and none reported symptoms of AN (according to a questionnaire).

Twenty-six patients (age 20-46 years, mean 31) had short to moderate duration of diabetes (5-19 years, mean 11) and 13 of these had developed retinopathy (at 6-15 years of diabetes, mean 11). The other 13 had no retinopathy but were closely matched with regard to sex and age. There were 6 female and 7 male pairs.

The remaining 26 diabetics (age 35-72 years, mean 55) had long duration of diabetes (21-49 years, mean 35) and 13 were without retinopathy. These patients were matched as regards sex and, as far as possible, age. This group also contained 6 female and 7 male pairs.

Controls. Two different control groups were used.

Group 1 (peripheral and autonomic nervous evaluation). A detailed report about this group has been given (7). In brief, diabetics of short duration were matched with 21 (7 females) young, healthy volunteers (age 28-48 years, mean 40) and the diabetics of long duration were matched with 24 (8 females) older, healthy volunteers (age 40-74 years, mean 53).

Group 2 (haemostatic evaluation). Fifty-three age-matched healthy volunteers were included in this group (age 25-74 years, mean 46). When comparing the results, diabetics of short duration were matched with 18 (10 females) of young controls (25-38 years, mean 32) and diabetics of long duration with the remaining 35 (12 females) older controls (40-74 years, mean 53).

Informed consent was obtained from all subjects.

Procedures

Neurological tests

Peripheral neuropathy. Examination of the nervous system included analysis

of tendon reflexes and vibration sense. Vibration sense thresholds (Biothesiometer) were determined over the medial malleoli. Absent ankle reflexes and/or abnormal vibration sense (above the mean of controls + 2 SD) were considered signs of PN (8).

Autonomic neuropathy. Three different tests were applied. Details about these tests have been reported (7,8,9). 1. Deep breathing test. The subject performed 6 maximal expirations and inspirations during 1 min of electrocardiographic (ECG) recording. The E/I ratio was calculated from the mean value of the longest R-R-intervals during expiration (E) and the shortest during inspiration (I). An E/I ratio below the lower range of controls was considered abnormal and a sign of vagal neuropathy (8). 2. Valsalva manoeuvre. The subject performed 3 Valsalva manoeuvres during ECG recording. The Valsalva ratio was calculated as a mean value from the 3 measurements of the ECG (the longest R-R-interval after manoeuvres to the shortest during the manoeuvres)(10). A Valsalva ratio below the lower range of controls was considered abnormal and a sign of mainly vagal neuropathy (8). 3. Orthostatic test. After 10 min of rest on a tilt table, the subject was tilted fast (2 s) head up (90°) during continuous ECG recording. The blood pressure was also recorded 4 min before tilting and then every minute after. The immediate heart rate reaction to tilting (an immediate acceleration followed by a transient deceleration) was described by the acceleration and brake indices (7). An acceleration or a brake index below the lower range of controls was considered abnormal and signs of vagal neuropathy although sympathetic denervation also influences the acceleration index (8). Greater falls in systolic or diastolic blood pressures 1 min after tilting than the range of controls were also considered abnormal and signs of sympathetic denervation (11). The indices could not be estimated in 3 diabetics of long duration because of frequent extra systoles (2 patients) and for other technical reasons (1 patient).

AN was considered to be present when one or more of the AN tests were abnormal.

Peripheral circulatory tests

Three different tests were applied in the following order.

1. Systolic blood pressure ratios. The systolic blood pressures were recorded in arms (indirect sphygmomanometer), ankles (ultrasound), and great toes (strain gauge technique)(12). Ankle/arm as well as toe/ankle pressure ratios were estimated. Ankle/arm pressure ratio <0.90 and toe/ankle pressure ratios <0.85 were considered abnormal (13). In one diabetic of long duration only recording of the left leg could be performed.

2. Venous occlusion plethysmography. Water plethysmography was performed according to Dahn (14). A first flow <14 ml/100 ml tissue/min or a maximal flow <17 ml/100 ml tissue/min was considered abnormal (15). Plethysmography could not be performed in one diabetic of long duration. In another diabetic of long duration only recording of the right leg could be performed.

3. Thermography. The infra-red emission was recorded with AGA Thermovision Equipment System No 661 (Stockholm, Sweden)(6). At first, the feet of the subject were immersed for 10 min in water with a temperature of 15°C. Thereafter, the subject lay down, the feet were wiped off and a heater was placed over the trunk during 40 min to provide indirect heating of the feet. At the start of indirect heating 15 g ethanol was given orally. The infra-red emission from the skin of the feet were recorded before and after cooling as well as every 10th min during the period of indirect heating. The mean

temperature of the toes of each foot was recorded. An increase in mean toe temperature of $<3^{\circ}\text{C}$ after 40 min of indirect heating was considered abnormal (6). Thermography was not performed in one diabetic of short duration.

Endothelial factors

In diabetics, blood samples and vessel wall biopsies from the dorsum of the hand were obtained 3 h after the morning dose of insulin (2 h after breakfast) on the day before the performance of AN and peripheral circulatory tests. In controls, blood samples were obtained postprandially after lunch.

Spontaneous fibrinolytic activity was measured by testing redissolved euglobulin precipitate of citrated plasma on unheated fibrin plates according to Nilsson and Olow (16) but human fibrin was used instead of bovine.

Fibrinolytic response to venous occlusion. The local release of plasminogen activator during artificial venous occlusion of the arms for 20 min was measured according to Robertson et al (17). Blood samples were drawn immediately before the cuff was deflated, and the fibrinolytic activity of resuspended euglobulin precipitate was determined on unheated fibrin plates.

Plasminogen activator activity of vascular walls was assessed from biopsy specimen of a superficial dorsal vein of the hands by a modification of the histochemical method of Todd (18). Normal range 6.0-10.0 arbitrary units of possible 12. Median value 7.5.

Factor VIII related antigen (VIIIIR:Ag) was quantitatively determined by electrophoresis of citrated plasma in agarose gel containing antibodies by rocket technique (19).

Statistical analysis. Endothelial factors and blood pressure ratios were tested by Mann-Whitney U-test. Differences in prevalence were tested by Fisher exact test. Otherwise unpaired t-test was used. All tests were two-tailed. $P < 0.05$ was considered significant. Results are given as mean $\pm$ SEM.

RESULTS

Autonomic neuropathy

Table I shows the prevalence of AN and the relationship to PN. In diabetics of short duration, the prevalence of AN was significantly higher in males than in females (9/14 vs 2/12, $p < 0.05$). No sex differences were found in long duration.

Peripheral circulation and endothelial factors

Diabetics of short duration. After cooling followed by heating the mean toe temperature was significantly lower in diabetics with AN than in those without (Table II, Fig. 1). Eight of 26 diabetics had an increase of toe temperature $\leq 3^{\circ}\text{C}$ and 7 of these had AN. Diabetics without AN showed significantly lower first flows as well as toe/ankle pressure ratios than those with AN. In fact, 11 out of 15 without AN had an abnormally low first flow, compared with only 3 out of 11 with AN. There were no differences in maximal flows or ankle/arm pressure ratios.

The plasminogen activator release during venous occlusion was significantly lower in diabetics than in controls and particularly low release was shown in

TABLE I

The Prevalence of Autonomic Neuropathy (AN) and the Relationship to Peripheral Neuropathy (PN) as well as to Retinopathy. Numbers between the Brackets give the Prevalence of PN in Diabetics with AN

	Diabetics of short duration			Diabetics of long duration		
	Without retinopathy	With retinopathy	All	Without retinopathy	With retinopathy	All
	(n=13)	(n=13)	(n=26)	(n=13)	(n=13)	(n=26)
PN	2	5	7	9	10	19
AN	4 (0)	7 (4)	11 (4)	7 (6)	10 (9)	17 (15)

TABLE II

Thermography, Plethysmography, and Blood Pressure Ratios in the Legs of Diabetics

	Diabetics of short duration		Diabetics of long duration	
	Without AN (n=30 ^a)	With AN (n=22)	Without AN (n=18 ^b)	With AN (n=34 ^c)
<u>Thermography (°C)</u>				
before cooling	26.3±0.5	27.0±0.9	26.8±0.5	28.0±0.5
after cooling	19.7±0.1	20.0±0.2	19.2±0.3	19.5±0.2
after 40 min of indirect heating	32.2±0.6	24.2±0.9***	28.4±1.0	27.2±0.9
<u>Plethysmography (ml/100 ml tissue/min)</u>				
first flow	12.7±0.7	17.0±0.7***	16.1±0.7	15.1±0.7
maximal flow	21.0±1.0	21.7±0.8	21.9±1.2	19.7±0.8
<u>Blood pressure ratios</u>				
ankle/arm	0.97±0.01	1.00±0.02	1.01±0.02	1.02±0.04
toe/ankle	0.86±0.02	0.96±0.02***	0.86±0.02	0.80±0.03

AN = autonomic neuropathy; n = number of legs. Results are given as mean ±SEM.

^an = 28 in thermography; ^bn = 16 in plethysmography; ^cn = 33 in plethysmography and blood pressure ratios, ***p<0.001 vs diabetics of short duration without AN

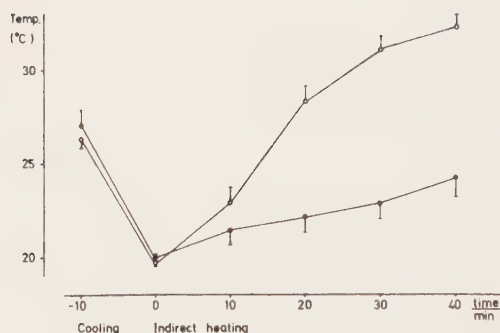


FIG. 1

Short diabetes duration

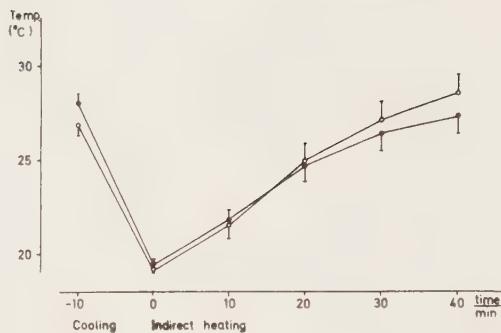


FIG. 2

Long diabetes duration

Mean toe temperature before and after cooling followed by indirect heating. Vertical bars = 1 SEM; o—o legs in diabetics without autonomic neuropathy (n=28); ●—● legs in diabetics with autonomic neuropathy (n=22).

those without AN (Table III). The plasminogen activator release in diabetics with a toe temperature increase $<30^{\circ}\text{C}$ did not differ from controls ($394 \pm 37 \text{ mm}^2$ lysis vs $420 \pm 20 \text{ mm}^2$ lysis; NS). The spontaneous plasminogen activator activity as well as the plasminogen activator activity of vessel walls showed no significant differences between the groups. VIIIR:Ag was significantly higher in diabetics than in controls but did not differ between diabetics with and without AN.

Diabetics of long duration. There were no significant differences in temperature reactions, flow measurements, or blood pressure ratios between diabetics with and without AN (Table II, Fig. 2). The mean toe/ankle pressure ratio, however, was significantly ($p < 0.001$) lower in AN diabetics of long duration than in AN diabetics of short duration. Ten diabetics showed a low toe temperature increase to heating ($\leq 30^{\circ}\text{C}$). Contrary to in short duration, the frequency of AN was not increased in patients with low temperature increases (7/10 vs 10/16). The blood flows were, however, significantly lower in these patients than in other diabetics of long duration (first flow $13.9 \pm 0.9 \text{ ml/100 ml tissue/min}$ vs $16.4 \pm 0.5 \text{ ml/100 ml tissue/min}$; $p < 0.05$ and maximal flow $18.0 \pm 1.0 \text{ ml/100 ml tissue/min}$ vs $22.0 \pm 0.8 \text{ ml/100 ml tissue/min}$; $p < 0.01$).

The plasminogen activator release during venous occlusion was significantly lower in diabetics without AN than in controls (Table III). The spontaneous plasminogen activator activity showed no differences. The plasminogen activator activity of vascular walls was significantly higher in diabetics with AN than in those without. VIIIR:Ag was significantly higher in diabetics, with and without AN, than in controls. VIIIR:Ag was also significantly ($p < 0.05$) higher in diabetics of long duration than in those of short duration.

DISCUSSION

Autonomic neuropathy is often found in diabetics, and in the present study almost half of the patients with a mean diabetes duration of 11 years (short duration) had one or more positive AN tests. Most of these patients showed a markedly delayed toe temperature reaction to indirect heating after cooling of the feet compared to those without signs of AN. The reason for the small

TABLE III

Physical Characteristics and Endothelial Factors in Diabetics and Controls

	Young controls	Diabetics of short duration			Older controls	Diabetics of long duration		
		all	without AN	with AN		all	without AN	with AN
n	18	26	15	11	35	26	9	17
Age (y)	32 $\pm$ 1	31 $\pm$ 1	30 $\pm$ 2	33 $\pm$ 1	53 $\pm$ 2	55 $\pm$ 2	52 $\pm$ 4	56 $\pm$ 2
Dur. (y)	-	11 $\pm$ 1	11 $\pm$ 1	11 $\pm$ 1	-	35 $\pm$ 1	34 $\pm$ 2	35 $\pm$ 2
Spont.pl.gen activator activity (mm ² lysis)	147 $\pm$ 11	119 $\pm$ 9	116 $\pm$ 13	124 $\pm$ 15	122 $\pm$ 7	123 $\pm$ 9	112 $\pm$ 18	128 $\pm$ 9
Pl.gen act. release (mm ² lysis)	420 $\pm$ 20	324 $\pm$ 21*	315 $\pm$ 22*	337 $\pm$ 40	432 $\pm$ 17	368 $\pm$ 17	347 $\pm$ 35*	379 $\pm$ 20
Pl.gen activator activity of vessel walls (U)	-	7.7 $\pm$ 0.3	7.4 $\pm$ 0.4	8.2 $\pm$ 0.5	-	8.0 $\pm$ 0.3	7.0 $\pm$ 0.6	8.6 $\pm$ 0.3 [†]
F VIII rel.antigen (%)	107 $\pm$ 11	137 $\pm$ 10*	143 $\pm$ 12	129 $\pm$ 15	135 $\pm$ 8	193 $\pm$ 15**	189 $\pm$ 25*	195 $\pm$ 19**

AN = autonomic neuropathy; n = number of subjects. Results are given as mean $\pm$ SEM. *p<0.05 vs controls, **p<0.01 vs controls, and [†]p<0.05 vs without AN.

increase in temperature (mean = 4.2°C after 40 min of indirect heating) in those with AN was most likely a prolonged vasospasm of the feet as blood flows and pressure ratios did not indicate vascular obstructions of the macrocirculation. The concept of vasospasm is further supported by the finding of normal blood flows and pressure ratios in the 8 patients of short duration with a toe temperature increase $\leq$ 3°C.

Only test abnormalities indicating parasympathetic damage were present in AN patients of short duration of diabetes (10). Accordingly, a disturbed autonomic nervous balance with unopposed sympathetic nervous activity might be responsible for the vasospasm, as suggested in our previous report (6). A sympathetic denervation hypersensitivity (1) cannot be ruled out however. The fact that injections of acetylcholine can abolish cold induced vasospasm (20) speaks in favour of a primary parasympathetic lesion.

Patients without AN and with a short duration of diabetes had abnormal first flows at plethysmography as well as abnormal toe/ankle pressure ratios, but normal ankle/arm pressure ratios. Thus the abnormal results at plethysmography could not be explained only by vascular obstructions. An abnormal vascular reaction to anoxia cannot be excluded and further investigations are needed.

Patients with a very long duration of diabetes (mean 35 years) showed no differences in thermography, plethysmography, and blood pressure ratios bet-

ween those with and without AN. Half of these patients were free from signs of retinopathy and thus they were highly selected. Their diabetic matches - having signs of retinopathy - can also be regarded as highly selected, having survived diabetes with only minor late complications.

Ten diabetics with long duration and with abnormal thermography had significantly lower blood flows at plethysmography than the other diabetics of long duration, while the prevalence of AN did not differ between these groups. Accordingly, the delayed temperature reaction probably was due to macroangiopathy rather than AN mediated vasospasm.

Several abnormalities were found in the synthesis and release of endothelial factors. In short as well as in long duration the plasminogen activator release during venous occlusion was lower in diabetics than in non-diabetic controls. This impairment of fibrinolytic capacity, however, was marked only in those without AN. Those with AN showed an almost normal plasminogen activator release, although the result in short duration was blunted by the fact that 4 obese diabetics [mean body mass index significantly higher than in controls (24.4 ± 0.6 vs 20.7 ± 0.5 ; $p < 0.01$) and than in the other diabetics ($21.2 \pm 0.7/21.8 \pm 0.6$; $p < 0.05$)] with AN but with normal thermography showed a very low release (246 ± 74 mm²). A low plasminogen activator release in obesity has been reported previously (21). The other 7 diabetics with short duration with AN had a normal plasminogen activator release during venous occlusion (389 ± 42 mm²). Similarly, in long duration the plasminogen activator release was normal in diabetics with AN while those without had a significantly lower release than the controls. Hence, the endothelial release of plasminogen activator seems to be related to the activity of the autonomic nervous system as we previously have suggested (6).

Glucose may pass freely over the endothelial cell membrane, and hyperglycemia might be responsible for the decreased endothelial release of the plasminogen activator in diabetics (5). The finding of a low plasminogen activator release in diabetics without AN and a normal in those with AN indicates that the endothelial plasminogen activator release returns to normal when AN appears. Most likely the mechanism behind is a disturbed autonomic nervous balance with a dominance of the sympathetic nervous system. Catecholamines are also known to enhance the plasminogen activator release from the vascular endothelium via β -receptors (22). Thus, an unopposed sympathetic nervous activity will actually lead to an improvement of the impaired fibrinolytic capacity. However, the present study not only shows that the plasminogen activator release mechanism may be stimulated, but also the fibrinolytic activity of the vascular walls. Thus AN seems to induce a normalisation of the synthesis as well as the release of the plasminogen activator. It is tempting to speculate whether this normalisation of the fibrinolytic system might have counteracted mechanisms leading to vascular obstructions.

Factor VIIIIR:Ag is most important for platelet adhesion. In a previous study (4) we have reported a mean elevation of 140 % in uncomplicated diabetics, and in this study the mean concentration was 137 % in diabetics of short duration, which was significantly higher than in controls. Although there was an insignificant increase of VIIIIR:Ag with age in controls, this increase was more marked in diabetics. In long duration the value increased to 193 %. VIIIIR:Ag was particularly high in patients with signs of macroangiopathy i.e. diabetics of long duration with abnormal thermography and low maximal flows (216 ± 23 % vs 135 ± 8 % in controls; $p < 0.01$). A correlation between macroangiopathy and VIIIIR:Ag has also been reported by others (23, 24, 25). The present study showed no correlation between AN and VIIIIR:Ag.

There were no significant differences between the groups in smoking habits, blood glucose or daily insulin doses that might explain the results.

Thus in these insulin dependent diabetics the plasminogen activator release

was lower in those without AN than in controls, while those with AN showed a normal plasminogen activator release as well as a higher plasminogen activator activity of the vascular walls than other diabetics. This might be related to the disturbed autonomic nervous balance. Interestingly, those with short duration of diabetes without AN showed significantly lower first flows and toe/ankle pressure ratios than the others. Further studies are necessary to elucidate the mechanisms behind this finding, and to evaluate the possibility that asymptomatic AN might induce a normalisation of the impaired fibrinolytic system in diabetes.

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To
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ABBREVIATIONS

AN	autonomic neuropathy
PN	peripheral neuropathy
ECG	electrocardiogram
VIIIIR:Ag	factor VIII related antigen
E/I	expiration/inspiration ratio
AI	acceleration index
BI	brake index

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INTRODUCTION

Background

Among systemic disorders, diabetes mellitus is the most common cause of damage to the autonomic nervous system (1). The autonomic nervous system controls the activity of the heart, blood vessels, glands, and smooth muscles throughout the body. The afferent fibers of the autonomic nervous system are mostly unmyelinated with cell bodies in the dorsal root ganglion of the spinal nerves and in the sensory ganglia of certain cranial nerves (2). The efferent fibers comprise two groups of pathways, the sympathetic and the parasympathetic group. The sympathetic, preganglionic fibers are usually short and synapse at distance from the effector organs to long, postganglionic fibers. On the contrary, the parasympathetic, preganglionic fibers are long and synapse near the effector organs to short, postganglionic fibers. Among sympathetic as well as parasympathetic efferent fibers, the preganglionic are mostly myelinated and the postganglionic unmyelinated (2, 3).

Various symptoms of autonomic neuropathy (AN) were reported in diabetics by the end of the 19th century (1, 4, 5 for reviews) but diabetic AN as an entity was first described by Rundles in 1945 (6). Disturbed oesophageal motility (7), gastric atonia (8, 9), nocturnal diarrhea (10, 11, 12), atonic bladder (13), and impotence (14, 15) are the classical symptoms of parasympathetic denervation while symptoms of sympathetic denervation comprise ejaculatory dysfunction (16, 17) and postural hypotension (18, 19, 20, 21). Later other symptoms of sympathetic denervation have been added as sudomotor disturbances with anhidrosis of lower body (22, 23, 24) and vasomotor disturbances with failure in temperature reactions (25, 26, 27, 28). A distinct symptom of parasympathetic damage is facial sweating while eating (gustatory sweating) (29). Sudden cardiorespiratory arrest in relatively young diabetics without cardiac arrhythmia or myocardial infarction has also been considered a specific feature of diabetic AN (30). As most of these episodes occurred during or after anaesthesia or bronchopneumonia, defective respiratory reflexes seem to be responsible (30). Signs of AN have been found in diabetics without symptoms of AN (31, 32).

Abnormalities in autonomic nervous function parallel dysfunction in the peripheral nerves (33). Peripheral neuropathy (PN) is usually found in

diabetics with symptoms of AN (34). Accordingly, pathogenetic factors that might explain PN could be responsible for the development of AN (1, 5) although there is at least one difference. Peripheral but not autonomic nervous function improves after treatment of diabetic ketoacidosis (35).

Segmental demyelination and axonal loss occur in PN (36, 37, 38). Histological studies by Fagerberg indicated that an angiopathy with vascular occlusions of the vasa nervorum might be responsible for the development of PN (39). That suspicion has not been confirmed by others (36, 37) until recently, when Timperley et al reported fibrin plugs in the vessel walls within nerves in diabetics with neuropathy (40). Accordingly, PN might be a consequence of microangiopathy. Metabolic disturbances related to hyperglycemia leading to an increased sorbitol concentration (41) and a decreased myoinositol concentration (42) in the peripheral nerves may cause nervous dysfunction as well. Lipid abnormalities of the myelin have also been discussed (43). Most recently, an increased glycosylation of the proteins of peripheral nerves has been found in diabetic rats and this might be another reason for PN (44).

Morphological studies of the autonomic nerves in diabetics have shown parasympathetic lesions in the vagal nerve with preganglionic demyelination (45, 46, 47) as well as damage to the parasympathetic nerves of the urinary bladder and the corpora cavernosa (48, 49). In the sympathetic nerves, preganglionic demyelination as well as lesions of the sympathetic ganglia have been observed (47, 50, 51, 52).

Evaluation of autonomic nervous function

Various noninvasive tests of AN, mostly measuring cardiovascular reflexes, have been developed during the last decade (1 for review). Thus, in 1973 Wheeler and Watkins (53) introduced the deep breathing test in the evaluation of AN. In this test the subjects breathe deeply while the heart rate is recorded through an automatic heart rate monitor. The difference between maximum and minimum heart rate is recorded and has been found to be low in diabetics with AN (53). The disadvantage is that an automatic heart rate monitor is needed. A deep breathing test using a conventional ECG would have a much wider application.

The Valsalva manoeuvre has been revived as a test of AN. Thus, again in 1973, Ewing et al (54) introduced the Valsalva ratio in the evaluation of AN (longest R-R-interval of the ECG after the manoeuvre to the shortest during). An abnormally low Valsalva ratio was considered a reliable sign of AN (54). The Valsalva ratio is reported to be reproducible (55) and has been widely accepted (56, 57, 58, 59).

The immediate heart rate reaction to standing up comprises a biphasic response with an initial acceleration followed by a transient deceleration (60). In diabetics with AN, a disturbed initial heart rate reaction to standing up was shown by Page and Watkins in 1977 (61) and confirmed and further evaluated by Ewing et al in 1978 (62). However, an active orthostatic test has obvious disadvantages. The patient cooperation has to be close and standardization of the test is difficult. A passive orthostatic test on tilt table ought to solve these problems but a previous attempt to evaluate AN from the initial heart rate reaction to tilting was without success (61).

The advantage of these recently developed tests is that AN can be measured quantitatively. Disturbed cardiovascular reflexes, however, are not necessarily directly related to all the various symptoms of AN. The relationships between abnormal cardiovascular reflexes on one side and retinopathy, PN, as well as the duration of diabetes on the other are also unclear.

Peripheral circulation and AN

Diabetic foot lesions are frequent in diabetics with PN (63). Sensory as well as motor disturbances are thought to be important for the development of such lesions (63). However, in 1966 and thus before the development of the recent AN tests, Moorhouse et al found abnormal vascular reactions to thermal stimuli in the feet of diabetics with PN (25). The finding was supposed to indicate increased vascular reactivity owing to sympathetic denervation. Accordingly, AN might contribute directly to the development of foot lesions. The possible correlation between AN and peripheral circulation needs to be evaluated by the recent AN tests.

Vascular occlusions are also important for the development of diabetic foot lesions (64, 65). The VIIIR:Ag, which is important for the platelet adhesion to vascular walls (66), is often increased in diabetics (67). On the contrary, the plasminogen activator activity, which is essential for an

adequate fibrinolytic activity, is low in many diabetics (68). Accordingly, a disturbed balance between coagulation and fibrinolysis is present in many diabetics favouring a development of vascular occlusions. Interestingly, the histological studies by Fagerberg (39) and Timperley (40) indicate that haemostatic factors could be of importance for the development of neuropathy. The VIIIR:Ag and the plasminogen activator are rapidly released from the endothelium of the vessel walls by suspect adrenoreceptor stimulation (69). Accordingly, sympathetic nervous activity may govern the release of these endothelial factors. The haemostatic balance may be changed by AN.

Aims of the present investigation

1. To develop a simplified deep breathing test using a conventional ECG.
2. To develop a standardized passive orthostatic test on tilt table.
3. To compare these tests with the results of the Valsalva manoeuvre as measured by the Valsalva ratio.
4. To evaluate autonomic nervous function in diabetic patients without symptoms of AN and correlate the results with the duration of diabetes, retinopathy, and PN.
5. To find out whether the degree of test abnormalities is increased in diabetic patients with symptoms of AN.
6. To find out whether there are differences between the AN tests in the evaluation of autonomic nervous function in diabetics with symptoms of AN.
7. To evaluate the relationship between AN and peripheral circulation.
8. To evaluate the relationship between AN on one side and VIIIR:Ag and the plasminogen activator on the other.

Subjects

Eighty-two insulin-dependent diabetic patients from a diabetic clinic comprising about 2000 patients were investigated. All patients were without clinical evidence of cardiac failure and had answered a questionnaire about positive symptoms of AN (postural or gastro-intestinal symptoms, gustatory sweating, bladder dysfunction, total impotence, or ejaculatory dysfunction). No patients were taking any medication apart from insulin.

Paper I. Forty-one diabetics without symptoms of AN (age < 50 years) and 25 age-matched healthy controls were studied.

Papers II, III, and V. Fifty-two diabetics (34 from I) without symptoms of AN were studied. Twenty-six patients had short to moderate duration of diabetes (< 20 years) and 13 of these had developed retinopathy. The other 13 had no retinopathy but were closely matched with regard to sex and age. There were 6 female and 7 male pairs.

The remaining 26 diabetics had long duration of diabetes (> 20 years) and 13 were without retinopathy. These patients were matched regarding to sex and, as far as possible, age. This group also contained 6 female and 7 male pairs.

Thirty-one healthy volunteers (21 from I) acted as controls in the nervous evaluation and 53 in the haemostatic evaluation.

Paper IV. Seventeen diabetics with symptoms of AN and 24 without but with PN (18 from II, III, and V) as well as 24 age-matched healthy controls (from II, III, and V) were studied.

In addition to subjects reported in papers I-V, 6 healthy volunteers (5 women) (age 21-32 years, mean 26) participated in an evaluation of the influence of the degree as well as the speed of rotation on the immediate heart rate reaction to tilting. A pharmacological study was also performed in 3 healthy men (age 24-31 years, mean 28).

Procedures

Retinopathy. All patients had their fundi dilated and underwent ophthalmoscopy, performed by the same experienced ophthalmologist. The finding of at least 3 microaneurysms was considered a sign of retinopathy.

Peripheral neuropathy (PN). Examination of the nervous system included analysis of tendon reflexes and vibration sense. Vibration sense thresholds (Bio-Thesimeter) were determined over the medial malleoli. The results were expressed in volts as the mean of measurements on the left and right ankles. In the control subjects, the threshold values increased with age and one 70 year old control subject had absent ankle reflexes. Threshold values above the mean of the controls + 2 SD were considered abnormal. Absent ankle reflexes and or abnormal vibration sense were considered signs of PN.

Deep breathing test. After 15 min in the supine position, the subject's heart rate was checked for at least 4 min and, once it was constant, a continuous ECG was recorded while the subject was taking deep breaths (6 maximal inspirations and expirations during 1 minute). The longest R-R-interval during each expiration and the shortest during each inspiration were measured. The mean values were calculated and the E/I ratio was calculated as follows:

$$E/I = \frac{\text{Mean value for longest R-R-intervals (s) during each expiration}}{\text{Mean value for shortest R-R-intervals (s) during each inspiration}}$$

Table I shows the reproducibility of the E/I ratio. The definition of abnormality was arbitrary (≤ 1.10) in paper I and below the mean of controls - 1SD in paper II. In the final evaluation, when the different AN tests were compared, an E/I ratio below the range of controls was considered abnormal; <1.10 in short duration and <1.09 in long duration (III,V) and <1.09 (IV).

Table I. The reproducibility of the E/I ratio, the acceleration index, and the brake index. Mean $\pm$ SEM. Second test performed one week after the first.

	E/I ratio (n=20) ^a	Acceleration index (n=25) ^b	Brake index (n=25) ^b
First test	1.24 $\pm$ 0.03	15.6 $\pm$ 1.7	16.2 $\pm$ 2.2
Second test	1.24 $\pm$ 0.03	16.8 $\pm$ 1.7	16.7 $\pm$ 2.4
Difference from the first test ^c	0.05 $\pm$ 0.01	3.9 $\pm$ 0.5	4.7 $\pm$ 1.0
r _s	0.92 ***	0.80 ***	0.82 ***

^a20 diabetics, ^b20 diabetics and 5 controls, ^cabsolute values of individual results, *** p<0.001.

Valsalva test. After 10 min of rest, the subject performed Valsalva manoeuvre (54, 70) by blowing through a mouthpiece attached to a manometer and maintaining a pressure of 40 mm Hg for 15 s. The Valsalva test was performed in the semirecumbent position. Once the technique had been mastered by the subject, the test was performed three times during continuous ECG recording. Between each blowing, the subject rested for 1 min. The Valsalva ratio was calculated from the ECG recordings (the mean longest R-R-interval after the 3 manoeuvres to the mean shortest R-R-interval during). Here, too, a ratio below the range of the controls was considered abnormal.

Orthostatic test. After the Valsalva manoeuvre was performed, the subject was fastened to a tilt table with straps around the upper parts of the chest, thighs and ankles with his feet on a bottom plate. To start with, the table was tilted head-up to familiarize the subject with the procedure. After that, the subject was returned to the supine position for 10 min and then was tilted to the upright position (90^0) in 2 s and remained so for 10 min. The rationale behind the 90^0 and 2 s tilt is discussed below. ECG was recorded continuously during the orthostatic test with start 1 min before tilting. Systolic and diastolic blood pressures were recorded indirectly in the right arm with a sphygmomanometer 4 min before tilting and then every minute.

Fig 1 shows the mean heart rate reaction during the first minute after tilting (90^0 and 2 s) in 6 healthy control subjects. The R-R-interval curve was characterized by a biphasic response with an immediate acceleration (= shorter R-R-intervals) soon followed by a transient deceleration (= longer R-R-intervals). When the curve was compared with those after lower degrees of tilting (70^0 and 50^0) (Fig 1) it was found that the most clear acceleration phase occurred after the 90^0 tilt. In addition, the deceleration phase was impaired after the 50^0 tilt. To evaluate the speed of rotation, the 6 control subjects were tilted 90^0 during 5 s, 10 s, and 20 s (Fig 2). The results showed that the 2 s tilt gave the most clear acceleration as well as deceleration phases. Accordingly, a 90^0 and 2 s tilt procedure was preferred for the evaluation of the immediate heart rate changes.

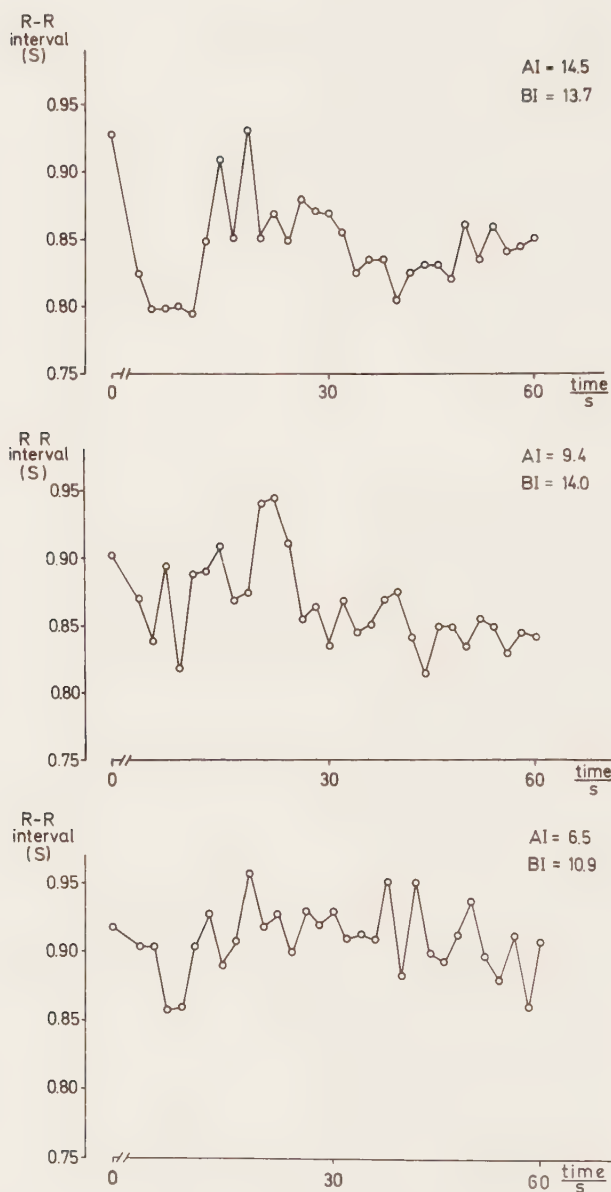


Fig 1. The influence of the degree of rotation on the immediate heart rate reaction to tilting in 6 control subjects. Mean values. AI = acceleration index. BI = brake index. At the top a 90° and 2 s tilt, in the middle a 70° and 2 s tilt, and below a 50° and 2 s tilt.

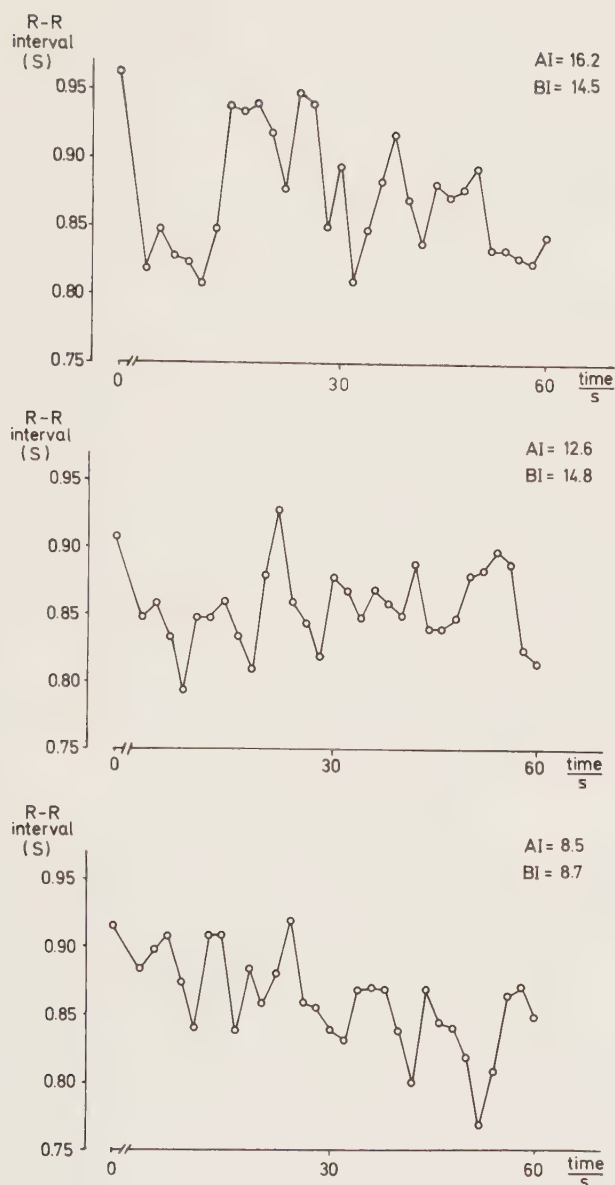


Fig 2. The influence of the speed of rotation on the immediate heart rate reaction to tilting in 6 control subjects. Mean values. AI = acceleration index. BI = brake index. At the top a 90° and 5 s tilt, in the middle a 90° and 10 s tilt, and below a 90° and 20 s tilt.

Calculations. The R-R-intervals were measured during the first 30 s of the last min before tilting and the mean value was taken as the R-R-interval at rest (A). During the first minute after tilting the shortest R-R-interval (B) before the transient deceleration was selected as well as the longest (C) after B. The mean R-R-intervals were then determined every minute. All R-R-intervals were expressed in seconds. To further clarify the initial changes $\frac{A-B}{A} \times 100$ (acceleration index) as well as $\frac{C-B}{A} \times 100$ (brake index) were estimated. To calculate the acceleration index in subjects with a brake index = 0, i.e. those without a deceleration, the shortest R-R-interval during the first 20 s after tilting (= time for shortest R-R-interval in controls + 2 SD) was selected and used as B. Table I shows the reproducibility of the indices. An index below the range of controls was considered abnormal.

Pharmacological study. To evaluate the nervous mechanism behind the heart rate reactions to deep breathing and to tilting, 2.5 mg atropine or 10 mg propranolol were given i.v. to 3 healthy men before the performance of the tests.

Peripheral circulatory tests comprised 1. systolic blood pressure ratios, 2. venous occlusion plethysmography, and 3. thermography. Details about the tests are shown in paper V.

Endothelial factors comprised 1. spontaneous fibrinolytic activity, 2. fibrinolytic response to venous occlusion, 3. plasminogen activator activity of vascular walls, and 4. VIIIR:Ag. Methods for analysis are shown in paper V.

Statistical analysis. Ratios, indices, and haemostatic factors were tested by Mann-Whitney U-test and Wilcoxon signed rank test (71). For correlations, the Spearman rank correlation coefficient was estimated (71). Differences in prevalence were tested by Fisher exact probability test or chi-square test. Otherwise unpaired and paired t-tests were used. $P < 0.05$ was considered significant. Results are given as mean $\pm$ SD, mean $\pm$ SEM, and range.

RESULTS AND DISCUSSION

Evaluation of the AN tests

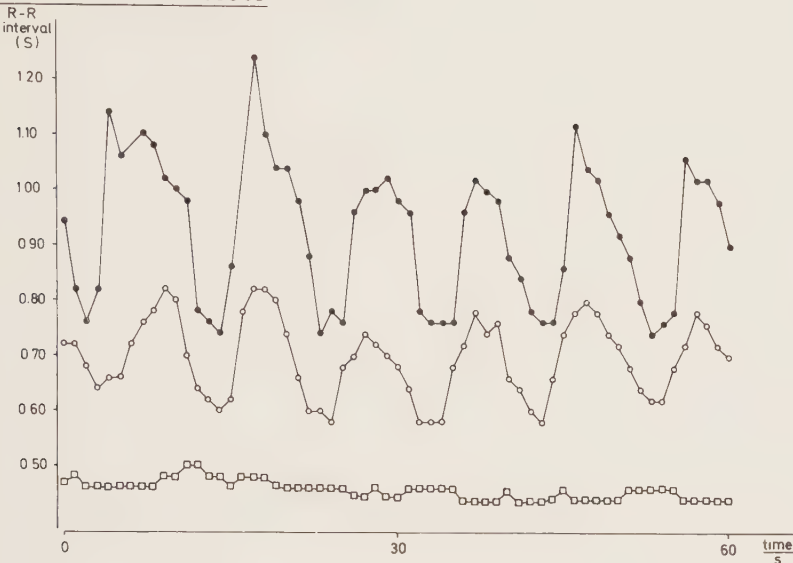


Fig 3. The heart rate variation during deep breathing in a control subject before (○), after propranolol (●), and after atropine (□). Similar patterns of heart rate variations occurred in the other 2 control subjects.

Deep breathing test. The heart rate reaction to breathing is characterized by an increase in heart rate (= shorter R-R-intervals) during inspiration and a decrease (= longer R-R-intervals) during expiration (Fig 3). The amplitude of these changes increases during deep breathing (72) and is maximal when 5-6 breaths are performed per minute (72, 73, 74). Inspiration is the main stimulus for the heart rate changes (75). The mechanism behind is not completely understood. Afferent stimulation from stretch receptors located within the chest as well as central factors have been suggested (72, 76). Recently, Melcher has emphasized the importance of hemodynamic factors (77). Inspiration increases the filling pressures of the heart and right ventricular stroke volume and secondarily, mediated by cardiac low-pressure receptors, the heart rate increases. Pulmonary reflexes only contribute slightly to the heart rate response during the end of the early phase of expiration. Primary central nervous factors are not involved. The efferent link is the vagal nerve (53) as also indicated from the atropine experiments (Fig 3).

The deep breathing test was introduced to evaluate vagal neuropathy (53) and abnormal results were shown in diabetics with symptoms of AN together with signs of PN. In the present investigation, unlike Wheeler and Watkins, the R-R-interval variation was evaluated through a conventional ECG and the differences in heart rate were estimated by the E/I ratio. The results showed that the E/I ratio was as efficient to detect AN as the original method (1). The close correlation between the 2 methods ($r=0.97$) has recently been confirmed (78).

The variation of R-R-intervals during quiet breathing has also been used as a test of AN (31, 32). Here, the variation of the R-R-intervals during quiet breathing, expressed as the SD of the mean resting R-R-interval, was evaluated (1) and found to be low in diabetics with low E/I ratios. However, periods of rapid heart rates unrelated to respiration with high SD values can occur in diabetics with obvious vagal neuropathy during quiet breathing (79). Furthermore, Ewing et al (78) have shown that the SD of the mean R-R-interval as well as the MSSD (= mean square successive difference), which is another way to express the variation (32), correlate significantly with the heart rate during quiet as well as deep breathing (high heart rates give low variations). Such a correlation was not shown when the difference between maximum and minimum heart rate was used to express the variation (78). The heart rate is increased in insulin-dependent diabetics as a group (1), perhaps because of insulin therapy (80), and can be very high even in patients without vagal neuropathy (81). Thus, although vagal neuropathy is a major cause of increased heart rates in diabetics (82), the frequently shown increased heart rates in other diabetics favour the E/I ratio instead of the "SD" for measuring vagal neuropathy. In the present investigation, the deep breathing test was performed in the supine position. Presumably, a test in the sitting or standing position had distinguished the patient groups better (78).

The deep breathing test showed that the E/I ratio decreased with age (Table II). Impairments occurred in those above 50 years in accordance with earlier reports (53, 83). This reflects a decrease in vagal tone with age (53). Obviously, age-matched controls have to be used in the evaluation of AN.

Table II. Heart rate ratios and indices in control subjects. Mean $\pm$ SEM.

	Controls <50 years (n = 21)	Controls >50 years (n = 10)	Significance
E/I ratio	1.31 $\pm$ 0.03	1.16 $\pm$ 0.01	p<0.001
Valsalva ratio	1.58 $\pm$ 0.08	1.49 $\pm$ 0.06	NS
Acceleration index	21.0 $\pm$ 1.3	10.7 $\pm$ 1.4	p<0.001
Brake index	18.7 $\pm$ 2.5	10.2 $\pm$ 0.8	p<0.05

Valsalva test. The Valsalva manoeuvre comprises an abrupt transient elevation of intrathoracic and intra-abdominal pressures provoked by straining. The response to the manoeuvre can be divided into four phases (84):

1. An initial rise of arterial pressure and reduction of heart rate immediately after the onset of straining.
2. A fall and later partial recovery of arterial pressure, together with an acceleration of heart rate, during the period of straining.
3. A sudden brief reduction of arterial pressure and elevation of heart rate immediately following the release of straining.
4. An elevation of arterial pressure above control levels together with slowing of heart rate.

The heart rate and blood pressure reactions during phase 4 are commonly used in the evaluation of autonomic nervous function (20, 54, 85, 86). The blood pressure reaction measures sympathetic activity mediated by cardiopulmonary receptors (84) and the heart rate reaction, as described by the Valsalva ratio, vagal nervous activity secondary to exposure of baroreceptors to increased blood pressure (87). Thus an absent bradycardia in phase 4 could reflect sympathetic as well as vagal denervation. Further, a normal response to the Valsalva manoeuvre requires an adequate cardiac function. The heart rate as well as the arterial blood pressure reactions to Valsalva manoeuvre are disturbed in patients with left ventricular failure (70, 88). Myocardial

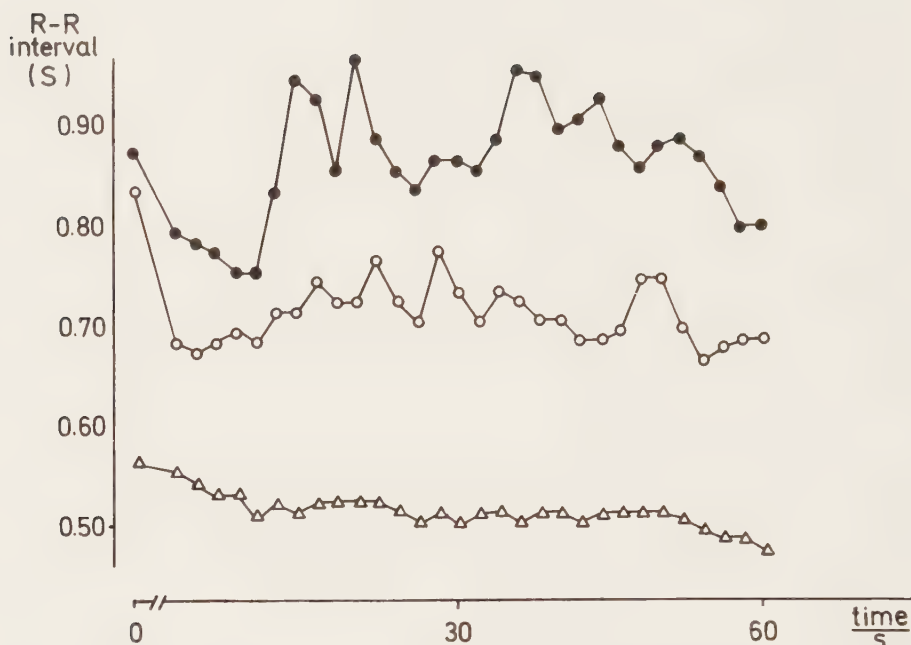


Fig 4. The heart rate reactions during the first minute after tilting in control subjects before (O), after propranolol (●), and after atropine (Δ). Mean values.

function as well as sympathetic and parasympathetic nervous functions have to be considered when the Valsalva ratio is evaluated. The Valsalva ratio has also been criticized (87, 89). Subjects with vagal neuropathy can have a tachycardia in phases 2-3 and thus falsely normal Valsalva ratios (87,89).

The reaction to the Valsalva manoeuvre decreases with age. Particularly the heart rate changes between phase 3 and 4 are impaired (90). In the present study the older control group tended to have lower values (Tab II). Perhaps the control sample was too small to detect age-dependent differences.

Orthostatic test. The normal heart rate reaction during the first minute after tilting is complicated and only partly understood. A head up tilt leads to pooling of the blood in lower parts of the body and a low pressure in carotid sinus (84). This stimulates baroreceptors to an immediate increase in heart rate, here estimated by the acceleration index, which has been attributed to withdrawal of vagal tone (91) as also supported from the atropine experiments (Fig 4). However, the initial acceleration was sluggish and delayed after propranolol suggesting an additional sympathetic influence.

Evidence for a minor adrenergic component in the immediate acceleration has also been presented by others (91). In addition, the immediate cardiac acceleration could have been elicited by the muscle-heart reflex (92, 93) as a slight plantar flexion might have occurred when the subjects reached the vertical position after the fast tilt (90^0 and 2 s).

The deceleration phase, here estimated by the brake index, seemed to be caused by reinstated vagal tone (atropine experiments) (Fig 4) which could be primary or secondary to sympathetic vasoconstriction which occurs 10-20 s after tilting (94).

A diastolic blood pressure rise after tilting as shown in healthy controls (II) has been reported earlier (95, 96, 97) and reflects sympathetic nervous activity (98). Baroreceptors are not the only receptors mediating the blood pressure response. Low pressure receptors are also involved (98, 99).

Instead of a standing up test a tilt table test was used to improve standardization. The immediate biphasic heart rate response was most clearly shown after the 90^0 and 2 s tilt. The failure to discover the immediate heart rate reaction in a previous study (61) might have been due to a lower speed or degree of tilting. Bräuer and Rosberg have also previously shown that a fast tilt (about 2 s) but not a slow gives a biphasic response similar to that after standing up (100). The importance of the speed of rotation was supported from the study of the 6 controls. In addition, the tilt angle seemed to be important. Particularly, the acceleration phase was impaired at lower angles. Previously, it has been shown that, in comparison to those at lower angles, a 90^0 tilt gives the most pronounced fall in pulmonary blood flow as well as stroke index (101). Probably, the marked haemodynamic consequences of a 90^0 tilt explain the intense acceleration.

To measure the immediate heart rate changes, an acceleration as well as a brake index was constructed. The brake index measured vagal activity as discussed and as supported from the close correlation with the E/I ratio (II). The acceleration index also measures vagal tone but, as discussed, an additional sympathetic influence cannot be excluded. In the formulae for the indices, the resting heart rate was considered. The 30:15 ratio (the 30:th R-R-interval to the 15:th R-R-interval), introduced in the standing up test (62), was estimated (II) but found without value after tilting. A similar conclusion about the 30:15 ratio has, however, been reported even after a standing up test (102).

The acceleration as well as the brake indices decreased in older subjects (Table II). This was not unexpected as cardiovascular reflexes decrease with age owing to decrease in venous compliance, increase in central blood volume, and reduction in baroreflexes (97).

Diabetics without symptoms of AN. Influence of duration of diabetes, retinopathy, and PN (I, II, III)

Deep breathing test. The deep breathing test discovered AN better than other methods. The efficiency of the test confirms the experience of others (34, 102). In diabetics of short duration the prevalence of an abnormal E/I ratio was higher in those with retinopathy than in those without (5/13 vs 0/13 and $p < 0.05$) (III). A similar correlation between retinopathy and vagal neuropathy has been reported recently (81, 102). However, no correlation was shown in diabetics of long duration (III). Thus, the duration of diabetes is also of importance for the development of vagal neuropathy. A reduced heart rate variation during breathing with an increased duration of diabetes has been reported by others (32, 102, 103).

The E/I ratio was low in diabetics of short as well as of long duration with PN (I, III). Accordingly, PN and vagal neuropathy were linked together. The prevalence of PN was high in long duration (III) and confirmed other reports (104, 105). Obviously, the prevalence of vagal neuropathy is high in patients with diabetes of long duration, too (III).

The relationship between vagal neuropathy on one hand and PN as well as retinopathy on the other is unclear. In short duration, retinopathy was present in all PN patients with an abnormal E/I ratio ($n=3$). Retinopathy was present in the other diabetics of short duration with abnormal E/I ratios ($n=2$) as well. Thus, retinopathy rather than PN seemed to be related to vagal neuropathy. Accordingly, vagal neuropathy might be a consequence of an angiopathy of the vasa nervorum. On the other hand, the close correlation between PN and vagal neuropathy and the absent between retinopathy and vagal neuropathy in diabetics of long duration indicate that metabolic factors related to an increased duration of diabetes also may give vagal damage.

Valsalva test (III). An abnormal Valsalva ratio occurred in 6 diabetics of long duration who showed PN and vagal neuropathy (= abnormal E/I ratios) as well. Consequently, the abnormal Valsalva ratio could have signed vagal neuropathy. However, 5 of the 6 patients also showed a diastolic blood pressure fall after tilting as a sign of sympathetic denervation. Hence, parasympathetic as well as sympathetic denervation were present in these patients. This well illustrates that an abnormal Valsalva ratio cannot distinguish sympathetic denervation from parasympathetic without additional tests (87, 89).

An abnormal Valsalva ratio without other AN test deviations occurred in 3 diabetics of short duration and 2 of them had signs of vasospasm in their feet after cooling (V). As such a vasospasm indicates a disturbed autonomic nervous balance - discussed in detail below - it is evident that even an isolated abnormal Valsalva ratio can be a sign of AN. The correlation between PN and impaired Valsalva tests in long duration confirmed previous observations (54, 86).

Orthostatic test. An impaired deceleration after tilting with an abnormally low brake index signed vagal neuropathy. In short duration, but not in long, the brake index was low in those with retinopathy (II). As the E/I ratio, the brake index was low in diabetics of short as well as of long duration with PN (III). Accordingly, as was discussed about the deep breathing test, the results of the orthostatic test also suggest that both microangiopathy and metabolic factors could be responsible for the development of neuropathy.

Five patients showed an abnormal brake index together with a normal E/I ratio. The discrepancy was not unexpected. Both measure vagal efferent function but the strength of the afferent stimulus differs. The brake index gives supplementary information about vagal function.

The immediate acceleration, here measured by the acceleration index, was low in PN although only in diabetics of long duration (III). This and the influence of age explain why the mean acceleration index was lower in diabetics of long duration than in those of short duration (II). It has been shown that baroreflex sensitivity is reduced in hypertension (106). Besides neuropathy, the elevated systolic blood pressure might have contributed to the low acceleration indices in diabetics of long duration with PN (III).

The increase in heart rate during the first minute after tilting was exaggerated in many diabetics (III) and might be a consequence of insulin therapy. Insulin injections enhance the increase in heart rate after tilting (107) and reduce plasma volume in diabetics without symptoms of AN (108). Recently, an exaggerated plasma adrenalin rise together with an exaggerated increase in heart rate on standing up has been shown in insulin-dependent diabetics (109).

The absent diastolic blood pressure rise after tilting (II) in diabetics with PN (III) confirms a previous report about a correlation between PN and sympathetic denervation (110). Efferent sympathetic nervous dysfunction has been shown in other long term diabetics (111, 112, 113).

Test results in diabetics with symptoms of AN and in those without but with PN (IV)

Deep breathing test. The prevalence of an abnormal E/I ratio was high in diabetics with symptoms of AN (77%) as well as in those with PN only (54%). The relationship between test deviations and symptomatic AN is difficult to evaluate because of the high frequency of an abnormal E/I ratio in those with PN only. In previous studies a high frequency of abnormal deep breathing tests has been shown in diabetics with symptoms of AN but most of these patients had PN (53, 114). Recently Mackay et al have published a large material of diabetics with symptoms of AN (n=64) showing a high frequency (84%) of abnormality in the deep breathing test (34). Again, most patients showed PN (60/64). Interestingly, the prevalence of abnormality was about the same as in paper IV although there were important differences in the materials. Most patients investigated by Mackay et al had parasympathetic symptoms (67% diarrhea) while sympathetic symptoms were predominating in paper IV (71%). In spite of this, the mean E/I ratio was slightly lower in patients with symptoms of AN than in those with PN only (IV). This suggests that parasympathetic neuropathy appears early in the development of AN and that the abnormality is increased when symptoms of sympathetic denervation appear. It has also been proposed, that parasympathetic denervation occurs before sympathetic (115). In the work of Mackay et al signs of vagal neuropathy as measured from the heart rate variation to deep breathing, were substantially lower in those with symptoms of AN (34). This strongly suggests, that the degree of test abnormalities increases when symptoms of AN occur.

Valsalva test. It was unexpected that the Valsalva ratio could not distinguish patients with symptoms of AN from those with PN only. Different cut-off values for abnormalities cannot explain the failure as a Valsalva ratio <1.18 , i.e. the definition of abnormality in paper IV, is borderline or abnormal in most studies (1, 54, 56, 57, 58). The patient selection might be responsible; perhaps more extensive vagal damage is needed for the appearance of abnormal ratios. Another possibility is the appearance of falsely normal Valsalva ratios which, as already discussed, can occur in patients with vagal neuropathy (87, 89). It is also possible that the high prevalence of an abnormal Valsalva ratio in a previous study (54) might have

reflected myocardial dysfunction rather than AN. Myocardial dysfunction, like neuropathy, is closely correlated with the duration of diabetes as well as with microangiopathy (116). Nevertheless, an abnormal Valsalva ratio signed extensive AN. All patients with an abnormal Valsalva ratio had an abnormal E/I ratio and an abnormal brake index.

Orthostatic test. The immediate heart rate reaction to tilting, especially the brake index, distinguished patients with symptoms of AN from those with PN only. Vasoconstriction induced by sympathetic nervous activity usually occurs 10-20 s after tilting (94). In AN, sympathetic denervation gives a disturbed vasoconstriction and vagal nervous tone already weakened by neuropathy is not reinstated. Mackay et al also reported a higher frequency of abnormalities in the deceleration after standing in diabetics with symptoms of AN than in those with PN only (57% vs 10% (34) and 82% vs 33% in paper IV). Interestingly, the difference in frequency of an abnormal immediate acceleration after standing up was about the same as the difference in frequency of an abnormal acceleration index after tilting (57% in diabetics with symptoms vs 13% in those with PN only (34) as compared with 53% vs 21% after tilting (IV)).

Thus, an abnormal heart rate reaction to tilting seems to be most specific for symptomatic AN. In AN, baroreceptor disturbances together with vagal and, presumably, sympathetic denervation give the impaired immediate acceleration while sympathetic denervation gives disturbances in the later acceleration, i.e. 10-20 s after tilting, as well as in the vasoconstriction. Most probably, the last mentioned disturbances are responsible for the appearance of postural symptoms but an adverse effect of an absent immediate acceleration cannot be excluded (117).

Endothelial factors, toe temperature, leg circulation, and AN (V)

After cooling followed by indirect heating, the toe temperature reaction was markedly delayed in many diabetics. Different mechanisms seemed to be causing the decreased circulation of the skin. In diabetics of short duration, the major factor was vasospasm because of a disturbed balance between parasympathetic and sympathetic nervous activity. In diabetics of long duration, more permanent vascular obstructions were operating.

In short duration, the first flows at plethysmography as well as the toe/ankle pressure ratios were lower in diabetics without AN than in those with. In spite of this, a markedly delayed toe temperature reaction occurred in those with AN. This suggests that a functional vasospasm caused the delayed temperature reaction in diabetics with AN.

In short duration, most diabetics with AN showed signs of parasympathetic denervation (III). Accordingly, a disturbed autonomic balance with unopposed sympathetic nervous activity might be responsible for the vasospasm. A sympathetic denervation hypersensitivity, however, cannot be excluded (25). An increased vascular reactivity to vasopressor substances has also been shown in certain diabetics (118). The observation that acetylcholine abolished cold-induced vasospasm in the fingers of diabetics (119) favours the concept of a primary parasympathetic lesion.

In long duration, only a slight delay in the increase of toe temperature occurred in diabetics with AN. These patients showed lower toe/ankle pressure ratios than AN diabetics of short duration. Accordingly, macroangiopathy might have blunted the AN related vasospasm. Both forms of macroangiopathy, atherosclerosis as well as media sclerosis, give impaired blood flows (120, 121) with ischemia that might counteract the tendency for vasospasm. The importance of macroangiopathy is further supported by the finding of low blood flows at plethysmography in long duration diabetics with abnormal thermography reactions (increase $< 3^{\circ}\text{C}$). Thus, abnormal thermography in short duration signed AN related vasospasm and in long duration extensive macroangiopathy.

The plasminogen activator release during venous occlusion was low in diabetics without AN and normal in those with. Hence, the release from the vascular endothelium was related to the function of the autonomic nervous system. The release of the plasminogen activator has been attributed to adrenoreceptors (69, 122) but other receptors are possible (123).

The normalized release might reflect an increased sympathetic nervous activity owing to parasympathetic denervation or sympathetic denervation hypersensitivity. An increased blood flow into the venous system over vascular endothelium during venous occlusion may in addition be responsible for the release of the plasminogen activator (124). In diabetics with AN, the vascular resistance might be reduced as a consequence of sympathetic denervation. If so, an increased blood flow reaches the venous vascular bed and secondarily the release of the plasminogen activator is increased. Evidence for arteriovenous shunting in the diabetic neuropathic foot has recently been presented (125, 126). Arteriovenous shunting in the arms should have the same effect on the release as a reduced peripheral resistance. In addition, venous occlusion might have induced an intense vasoreaction, like that in the feet during cooling, squeezing out the plasminogen activator from the endothelium in diabetics with AN.

A disturbed fibrinolysis has been reported in non-diabetics with severe atherosclerosis (127, 128, 129) and in diabetics with asymptomatic arterial insufficiency (130). The normalized fibrinolysis might have prevented the development of vascular occlusions in diabetics of short duration with AN. The importance of the fibrinolytic system for the resistance against macroangiopathy is also supported from the findings in long duration where a particularly low plasminogen activator release was shown in diabetics with impaired blood flows i.e. abnormal thermography responders ($332 \pm 21 \text{ mm}^2$ lysis vs $432 \pm 17 \text{ mm}^2$ lysis in controls, $p < 0.01$).

In contrast to the plasminogen activator release, the VIIIR:Ag concentration was not correlated with the presence of AN. This supports the concept that different receptors are responsible for the release of these two factors (123) and is a further evidence for the importance of vascular blood flow in the release of the plasminogen activator (124).

Diabetics of short duration showed a rise of VIIIR:Ag not related to vascular complications. The VIIIR:Ag then rose with increased duration of diabetes. A correlation between macroangiopathy and VIIIR:Ag has been reported (131, 132, 133). Here VIIIR:Ag was especially high in long duration patients with extensive macroangiopathy i.e. abnormal thermography responders. In addition, these patients, as discussed, showed a markedly disturbed fibrinolysis. Accordingly, there was a disturbed haemostatic balance in these patients favouring the development of vascular lesions.

GENERAL REMARKS

The findings of extensive AN test abnormalities in some of the diabetics without symptoms of AN show that advanced AN is more frequent than previously thought. When symptoms of AN are reported, most test results will be markedly impaired and it is difficult to quantitate the degree of autonomic nervous involvement. Most probably, the major field of application of the tests is the detection of early, minor disturbances in autonomic nervous function.

The AN tests cannot be performed in patients with cardiac arrhythmia. Even sinus tachycardia impairs the test results and particularly the acceleration and the brake indices are reduced. An increase in resting heart rate up to 130 beats per minute, however, may reflect vagal neuropathy (83).

The prognosis in diabetics with symptoms of AN is bad (134) but does not necessarily reflect AN only. Diabetics with symptoms of AN often have other complications, like nephropathy, that influence the prognosis. Nevertheless, about half the deaths in diabetics with symptoms and signs of AN are sudden and unexpected and most probably associated with AN (134). On the other hand, the prognosis is unknown in diabetics with asymptomatic AN (1). The present investigation was not designed to evaluate the consequences of asymptomatic AN although several observations point to an adverse effect of asymptomatic AN. The cold-induced vasospasm in the feet as well as the normalized plasminogen activator release during venous occlusions of the arms indicate that vascular hyperreactivity might be a general phenomenon in AN. Vascular hyperreactivity has been reported to be responsible for abnormal vaso-reactions to CO_2 in cerebral vessels among diabetics (135). An increased prevalence of variant angina suggests that coronary vasospasm is frequent in non-diabetics with Raynaud's phenomenon (136). A similar coronary vasospasm may occur in diabetics with AN. The elevated heart rates in diabetics with AN point to an increased O_2 consumption of the myocardium (137). Catecholamine, glucagon, and pancreatic polypeptide responses to various stimuli are impaired in diabetics with AN (110, 138, 139) and may give metabolic dysregulation.

The normal fibrinolysis in diabetics with AN counteracts a development of fibrin plugs in the vasa nervorum and does not entertain the suspicion (40) that a disturbed fibrinolysis causes neuropathy. Similarly, a disturbed haemostatic balance secondarily to neuropathy as cause of vascular occlusions seems unlikely. On the contrary, the normal fibrinolysis and peripheral circulatory tests in diabetics of short duration with AN suggest that AN might be a defence against macroangiopathy. However, despite the favour of a normal fibrinolysis, the AN related cold induced vasospasm might give disturbed perfusion in smaller vessels and be a link between neuropathy and diabetic foot lesions.

Cigarette smoking has been considered important in the development of retinopathy (140) and nephropathy (141). An adverse effect of cigarette smoking on vagal nervous function was suggested in paper I. Unfortunately, this could not be further evaluated in papers II, III, and V, as most patients of short duration were or had been smokers (21/26). However, the remaining patients of short duration (n=5) who never had smoked showed completely normal results in all 3 AN tests.

The prevalence of AN was increased in men with diabetes of short duration (V). An increased susceptibility to AN might explain the unfavourable prognosis among men with diabetes mellitus (142, 143).

Further studies are needed to evaluate the consequences of asymptomatic AN.

GENERAL CONCLUSIONS

1. The deep breathing test with estimation of the E/I ratio from a conventional ECG is a reliable test of vagal neuropathy.
2. The immediate heart rate reaction to a 90^0 and 2 s head up tilt comprises an initial acceleration followed by a transient deceleration. The acceleration as well as the brake index measure these changes in heart rate. Both indices evaluate mainly vagal nervous activity although sympathetic activity influences the indices in addition. An absent diastolic blood pressure rise after tilting signs sympathetic denervation.
3. The Valsalva ratio cannot separate parasympathetic neuropathy from sympathetic. Most patients with an abnormal Valsalva ratio have parasympathetic as well as sympathetic denervation.
4. Vagal neuropathy appears in asymptomatic patients and the prevalence increases with the duration of diabetes. Vagal neuropathy correlates with the prevalence of retinopathy in diabetics of short duration but not in those of long duration. There is a close correlation between vagal neuropathy and PN and about half of the patients with PN and without symptoms of AN show vagal neuropathy.
5. The degree of test abnormalities is increased in patients with symptoms of AN. The brake index is particularly low in those patients.
6. In comparison to other tests, deviations in the orthostatic test seem to be closely correlated with symptomatic AN. Most probably, sympathetic denervation contributes to the extremely low values of the brake index in patients with symptoms of AN.

7. A markedly delayed toe temperature increase to cooling followed by indirect heating occurs in diabetics of short duration with AN. Organic vascular occlusions are often absent in these patients. Accordingly, AN seems to favour a cold-induced functional vasospasm in diabetics of short duration.

8. The plasminogen activator release during venous occlusion is low in diabetics without AN and normal in those with. The VIIIR:Ag concentration does not correlate with the prevalence of AN. A disturbed autonomic balance with hyperreactivity of the vessels normalizes the plasminogen activator release from the endothelium.

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